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Dominance and Growth Factors of *Pseudanabaena* sp. in Drinking Water Source Reservoirs, Southern China

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Abstract: *Pseudanabaena* sp. is a common and harmful species in freshwater cyanobacteria blooms. There are very few studies on its distribution characteristics and growth influencing factors. In the current study, it was found to be dominant in three cascading reservoirs in Southern China. Field observations and laboratory experiments were integrated to investigate the dominance and growth factors of *Pseudanabaena* sp. The effects of temperature, light intensity, nutrients, chemical oxygen demand (COD), pH, and disturbance on *Pseudanabaena* sp. growth were evaluated. The results indicated that *Pseudanabaena* sp. had significant positive correlations with water temperature, pH, and COD ($p < 0.01$) and a positive correlation with NH_3-N ($p < 0.05$). The optimum growth temperature range for *Pseudanabaena* sp. was from 20 to 30 °C; hence, it usually has outbreaks in May and August. The optimum light intensity and pH for *Pseudanabaena* sp. were 27 μmol photons $m^{-2}s^{-1}$ and from 7 to 9, respectively. The superior tolerance for low light, disturbance, and phosphorus deficiency of *Pseudanabaena* sp. may be the main factors affecting its dominance in reservoirs. Controlling nitrogen was more effective than controlling phosphorus to avoid the risk that was brought by *Pseudanabaena* sp. This study contributed to the theoretical knowledge for the prediction and control of the growth of *Pseudanabaena* sp.

Keywords: *Pseudanabaena* sp.; dominance; growth factors

1. Introduction

Water, especially drinking water safety is an important guarantee for the realization of sustainable economic and social development. Seasonal cyanobacteria outbreaks in drinking water sources have become a severe issue around the world and have drawn considerable attention in recent years [1–3]. Rapidly proliferating cyanobacteria are one of the most significant causes of source water deterioration [4,5] and are a serious threat to the drinking water treatment processes as they can plug the filtration tanks/membranes and produce cyanobacteria organic matters [1,6,7]. Cyanobacteria organic matters, including microcystins, taste and odor compounds, and precursors of disinfection by-products, have been reported to be difficult to fully remove by conventional drinking water treatment [8,9]. Therefore, effective control of cyanobacteria in drinking sources is extremely important for drinking safety and human health.

Cyanobacteria usually dominate phytoplankton communities in eutrophic drinking water sources worldwide [5,10,11], and *Pseudanabaena* sp. is a common species in freshwater cyanobacteria blooms [12,13]. During the last few decades, the occurrence of *Pseudanabaena* sp. has been recorded

nearly all over the world [12–18]. Under adaptive conditions, *Pseudanabaena* sp. may exceed the other larger species in terms of biomass and activity and, therefore, has played a major role in the ecosystem dynamics [4,19,20]. As early as 1984 [14], *Pseudanabaena* sp. found in two different Southern California reservoirs were proven to be 2-methylisoborneol (MIB) producers. Subsequently, several reports illuminated that besides producing MIB, *Pseudanabaena* sp. could also produce cyanotoxins, including hepatotoxins and neurotoxins [13,17,21–23].

The above studies demonstrated that *Pseudanabaena* sp. may be a more widespread problem in drinking water than has heretofore been recognized, and this should not be neglected. However, until now, little attention has been paid to the growth characteristics of *Pseudanabaena* sp. Generally, the principal factors affecting the growth of cyanobacteria are the water temperature, pH, light conditions, nutrient concentrations, COD, and predation by zooplankton and fishes [4,11,24]. Several studies documented the effects of light and temperature on the growth and MIB production of *Pseudanabaena* sp. [18,25]. In a lake with *Pseudanabaena* sp. dominance, it was reported that there was no stronger depletion of soluble reactive phosphorus concentrations [26]. Nevertheless, the environmental factors affecting the *Pseudanabaena* sp. dominance have not been clarified. It is therefore meaningful to investigate all of the influential factors systematically in order to provide a better understanding of the dominance of *Pseudanabaena* sp.

The cascading reservoirs Xili-Tiegang-Shiyan are located in the highly developed Shenzhen City of Southern China. As essential components of the Dongjiang Water Source Project, the cascading reservoirs have an effective capacity of 124 million m^3 and a daily inflow of approximately 2 million m^3 from the Dongjiang River, ensuring the daily water demand of 5 million west Shenzhen residents. Shenzhen is one of the seven cities in China suffering from severe water shortages. Nearly 80% of the drinking water source depends on the interbasin water transfer project. Therefore, the water quality protection of the drinking water source reservoirs is essential for Shenzhen's sustainable development. Recently, the cascading reservoirs have experienced eutrophication problems as a result of the declining water quality in the Dongjiang River, as well as point and diffuse pollution sources in the watershed. In this study, a year-long monitoring project from June 2013 to June 2014 was carried out and *Pseudanabaena* sp. dominance was detected. Meanwhile, complaints about an earthy-musty taste in finished drinking water caused by MIB were raised by the residents utilizing the cascading reservoirs as their drinking water source. Consequently, *Pseudanabaena* sp. was speculated to be relevant to these events.

In this study, the results of both field observations and laboratory experiments were evaluated. The objectives were: (1) to describe the variation characteristics of environmental factors affecting the growth of phytoplankton in the cascading reservoirs; (2) to determine the temporal and spatial distribution of *Pseudanabaena* sp. in the cascading reservoirs; (3) to identify the interaction between *Pseudanabaena* sp. growth and environmental factors; and (4) to analyze the reason for the dominance of *Pseudanabaena* sp. in the cascading reservoirs. The results may provide useful theoretical information on the subsequent research of *Pseudanabaena* sp. This study may also pave the way for the establishment of effective strategies and measures for *Pseudanabaena* sp. control.

2. Materials and Methods

2.1. Study Area and Sampling Sites

The cascading reservoirs monitored in this study are located in Southern China (east longitude 113°52′–113°57′, north latitude 22°36′–22°42′; Figure 1). The Xili Reservoir was built in 1960. Encompassing a collection area of 29 km^2, the Xili is the uppermost cascade reservoir. The total storage capacity and normal storage capacity of the Xili Reservoir are 32.38 million m^3 and 24.82 million m^3, respectively. The Tiegang Reservoir was created in 1957 and has been expanded four times. The Tiegang encompasses a collection area of 64 km^2, with total storage and normal storage capacities of 99.50 million m^3 and 94.00 million m^3, respectively. The Shiyan Reservoir, the last cascade reservoir,

encompasses a collection area of 44 km^2. The total storage capacity and normal storage capacity of the Shiyan are 32.00 million m^3 and 16.90 million m^3, respectively. According to the competent water authority's historical data from 2010 to 2014, the Xili Reservoir received an average daily inflow of 1.06 million m^3 from the Dongjiang River and delivered 1.04 million m^3 per day to the Tiegang Reservoir via artesian flow. The Tiegang Reservoir transported 0.72 million m^3 per day to the Shiyan Reservoir through the Tieshi pump station.

Sampling was performed at nine sites following the water diversion route from Jun 2013 to Jun 2014 (Figure 1). Water temperature (T), pH, the dissolved oxygen concentration (DO), and turbidity were measured in situ with a YSI 6600 V2 Multi-parameter Water Analysis Instrument, Xylem, Yellow Springs, Ohio, OH, USA. Light intensity and transparency (SD) were measured with an LI-1400 illuminometer, LI-COR, Lincoln, Nebraska, NE, USA and a Secchi disc, respectively. The velocity distribution was measured with a RiverSurveyor M9 Doppler current meter, Xylem, USA. A sample of approximately 500 mL of water was taken from each site to measure nutrients and organic matter content. Quantitative samples of the phytoplankton were collected using 20 L of raw water samples concentrated to approximately 50 mL through a 25# plankton net (mesh diameter of 64 μm) in 100 mL vials for subsequent classification and counting under a microscope in the laboratory. At each sampling site, three layers—surface (20 cm below the water surface), middle, and bottom (10 cm above the sediment)—were considered.

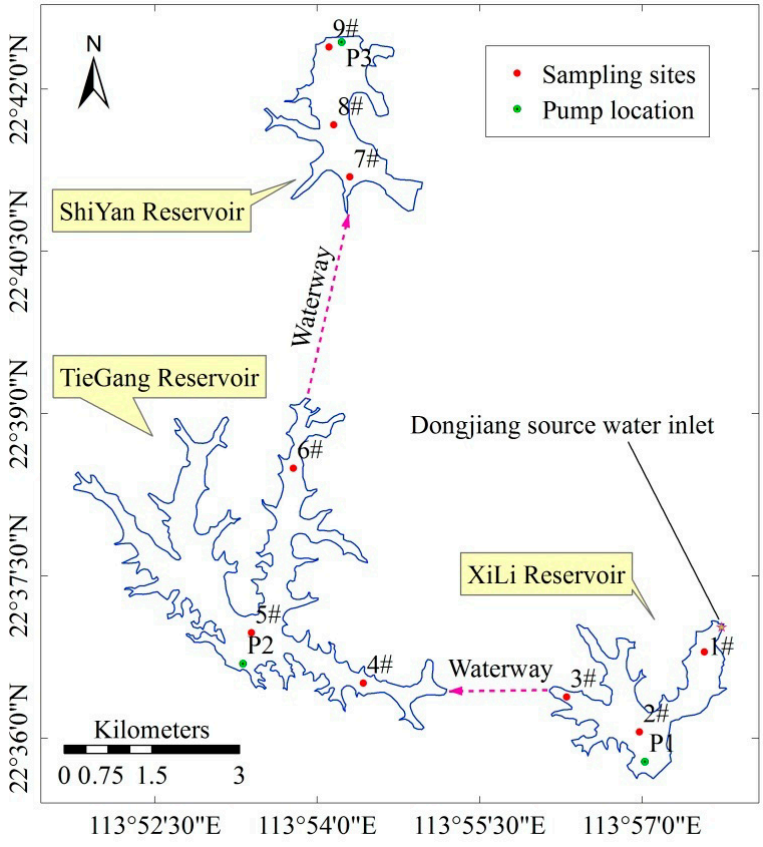

Figure 1. The locations of the cascading reservoirs and sampling sites.

2.2. Organism and Culture Conditions

The *Pseudanabaena* sp. used in this article was isolated from the sampling site #2 of Xili Reservoir by capillary separation (Figure 2). The cyanobacteria were cultured in 500 mL Erlenmeyer flasks each containing 300 mL of the BG11 medium under a temperature of 25 °C and illumination of 71.28 μmol photons m^{-2}s^{-1} using cool white fluorescent lights with a 12:12 light/dark cycle. The cultures of *Pseudanabaena* sp. in the exponential phase were collected by centrifugation and used as inoculants

for the different experiments. However, single factor tests in this study were conducted in batches. There were still subtle differences in the growth stages of inoculated algae for each batch, which may lead to differences in the growth rates of the algae in different batches of experiments.

The modification of the culture conditions was done according to the experimental design.

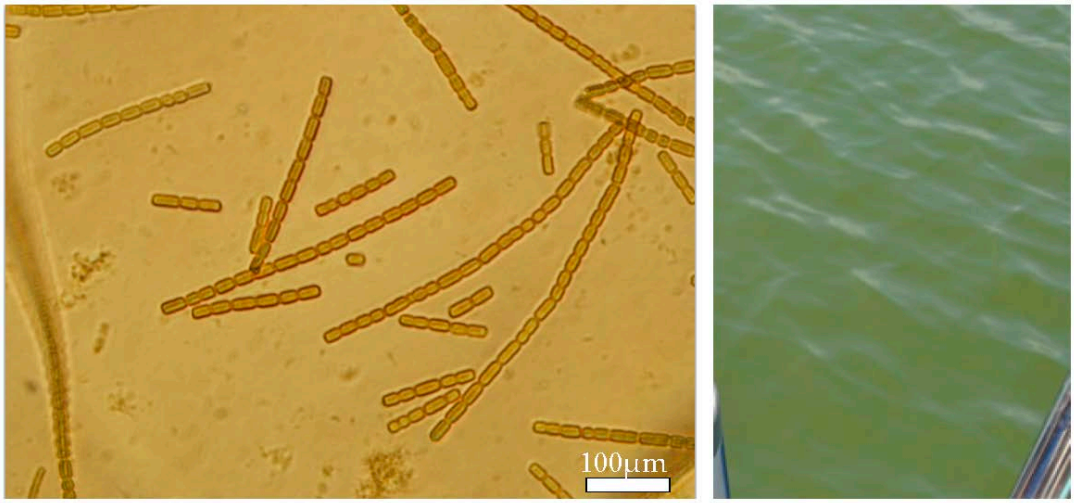

Figure 2. (**left**) The micro-morphology of *Pseudanabaena* sp. (**right**) The bloom in the cascading reservoirs.

2.3. Experimental Design

Six environmental parameters including T, illumination, disturbance, pH, total nitrogen (TN), and total phosphorus (TP) were investigated through laboratory experiments. All experiments were performed in 500 mL Erlenmeyer flasks containing 300 mL of culture, and the initial biomass evaluated by OD_{665} for each flask was adjusted to approximately 0.02. Each experiment lasted for 16 days and 3 mL of culture was sampled daily for the measurement of chlorophyll a (Chl a). The Erlenmeyer flasks were manually shaken and changed position randomly three times a day. The effect of the six different temperatures (ranging from 15 °C to 40 °C) and seven different illuminations (ranging from 0 to 216 μmol photons $m^{-2}s^{-1}$) on *Pseudanabaena* sp. growth was examined. The temperature and illumination were controlled by five Yiheng MGC-100P Incubators, Shanghai, China. To simulate the effects of various disturbances on the *Pseudanabaena* sp. growth, six IKA COLOR S025 magnetic stirrers, IKA, Staufen, Germany, with cylinder bars of 4 cm in length and 0.4 cm in diameter were used. Stirring speeds were set to be 0, 100, 200, 250, 300, 400, and 500 r/min, simulating the natural velocities in the reservoirs as 0, 0.02, 0.04, 0.05, 0.06, 0.08, and 0.10 m/s, respectively [27].

In the six experiment groups, one factor was changed while other factors were kept in the following values: temperature was 25 °C, illumination was 71.28 μmol photons $m^{-2}s^{-1}$, pH was 7, concentrations of TN and TP were 247.00 mg/L and 7.12 mg/L, respectively, no disturbance.

To explore the effect of pH on *Pseudanabaena* sp. growth, cultures were prepared in two treatment groups with initial pH gradients designed as follows: 3, 5, 7, 8, 9, and 11. For one group, the Chl a and pH were determined every day without pH adjustment. Meanwhile, for the other group, the Chl a and pH were determined every day and then the pH was adjusted to the initial value through the addition of hydrochloric acid or sodium hydroxide with a concentration of 0.1 mol/L.

Seven TP and TN concentration gradients (TN: ranging from 0 to 76.8 mg/L; TP: ranging from 0 to 10.24 mg/L) were designed to evaluate the correlation between *Pseudanabaena* sp. growth and nutrients. The TP and TN concentrations were adjusted by adding K_2HPO_4 and $NaNO_3$, respectively.

2.4. Analytical Methods

TP was analyzed with the ammonium molybdate spectrophotometry method. TN, ammonium (NH_3-N), and nitrate (NO_3-N) were analyzed using an AMS Alliance Futura Continuous Flow

Analyzer, Alliance, France. COD was determined with the permanganate titration method. The phytoplankton samples were counted directly in a blood counting chamber (0.0025 mm^2, 0.10 mm) using an Olympus BX-51 microscope at an objective magnification of 40×. Phytoplankton species were identified according to Freshwater algae in China [28] and Freshwater microbiological atlas [29] through software named Shineso Algae Analytic System, Shineso, China. Chl a concentrations were analyzed by a method based on acetone extraction and determination by spectrophotometry [18].

2.5. Statistical Analysis

In this study, the dominance value Y of *Pseudanabaena* sp. was calculated through Equation (1) [30]:

$$Y = (n/N) \times f \quad (1)$$

where n is the monthly mean number of *Pseudanabaena* sp. cells at the sampling sites in the whole year, N is the monthly mean number of the total phytoplankton cells at the sampling sites in the whole year, and f is the occurrence frequency of *Pseudanabaena* sp. that is calculated by the ratio of the number of samples with *Pseudanabaena* sp. to the number of samples at the sampling site in the whole year.

Each experiment included triplicate treatments. Data processing and graphing were performed with Excel 2010, and the analysis of variance (ANOVA) and correlation analysis were performed with SPSS 19.0 software.

3. Results and Discussion

3.1. Variations of the Environmental Factors

The concentration ranges and averages of the water quality variables in the cascading reservoirs from June 2013 to June 2014 are shown in Table 1. The transparency, which was measured by Secchi depth, varied from 0.40 m to 1.80 m. The monthly mean transparency of the Shiyan Reservoir was lower than that of the Xili and Tiegang reservoirs. The water temperature of the cascading reservoirs ranged from 13.3 to 32.7 °C, and the monthly average water temperature of the three reservoirs was 21.9, 24.1, and 24.3 °C. The light intensity in the reservoirs was characterized by a large vertical gradient. Taking sampling site #2 for example, the monthly average light intensities of the bottom, middle, and surface layers were 0.18, 121.17, and 271.30 μmol photons $m^{-2}s^{-1}$, respectively. Meanwhile, the light intensity of the three reservoirs varied from 0 to 709 μmol photons $m^{-2}s^{-1}$. The monthly average concentrations of TN in the three reservoirs were 1.72, 1.50, and 1.99 mg/L. The lowest concentration of TN (1.12 mg/L) occurred in the Tiegang Reservoir, and the highest occurred (3.06 mg/L) in the Shiyan Reservoir. NO_3-N was the dominant form of nitrogen, with an average ratio over 70%. Concentrations of NH_3-N were relatively lower (ranging from 0.02 to 0.73 mg/L). The monthly mean concentrations of TP in the three reservoirs were 0.02, 0.03, and 0.04 mg/L, and the highest concentration of TP reached 0.08 mg/L in the Xili and Tiegang reservoirs. Combining the TN and TP concentrations of each sampling, the N:P ratio in the cascade reservoirs was mostly above 16, which indicated that the cascade reservoirs are P-limited, according to Redfield's (1958) quantification. The biomass of the total phytoplankton in the cascading reservoirs was up to 9.23×10^7 cells/L, a biomass that is above the alert level 3 (5.00×10^7 cells/L) defined by Izydorczyk et al. [8]. The phytoplankton was mainly composed of cyanobacteria, chlorophyte, and diatom. The dominant species included *Leptolyngbya* sp., *Raphidiopsis* sp., *Pseudanabaena* sp. and *Merismopedia* sp., as shown in Appendix A. The maximum biomass of *Pseudanabaena* sp. also reached 3.56×10^7 cells/L in the Shiyan Reservoir.

Following the water diversion route (Xili-Tiegang-Shiyan), the water quality deteriorated gradually and was manifested by the decline of the mean transparency and the rise of COD_{Mn}, NH_3-N, TP concentrations, and the biomass of total phytoplankton and *Pseudanabaena* sp. After performing an ANOVA on the water quality parameters in the three reservoirs (Table 2), significant spatial differences of the total phytoplankton cells (Tcells), *Pseudanabaena* sp. cells (Pcells), pH, COD_{Mn},

NH_3-N, TN, and TP were found ($p < 0.001$). Turbidity, T, and DO had no significant differences in any of the locations ($p > 0.05$). The seasonal differences of T, DO, COD_{Mn}, and TN were statistically significant ($p < 0.001$). Meanwhile, the total phytoplankton cells were observed to be closely related with the seasons ($p < 0.001$). There were no significant correlated relationships between the other parameters and seasons ($p > 0.05$).

Table 1. The environmental variables in the cascading reservoirs.

	Xili (Sites #1, #2, #3)			Tiegang (Sites #4, #5, #6)			Shiyan (Sites #7, #8, #9)		
Parameters	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**	**Min**	**Max**	**Ave**
SD (m)	0.40	1.80	0.83	0.50	1.60	0.83	0.50	1.30	0.68
T (°C)	13.7	31.5	21.9	14.2	31.6	24.1	13.3	32.7	24.3
Illumination (μmol photons $m^{-2}s^{-1}$)	0.00	709.00	152.29	0.03	601.30	153.29	0.03	405.80	123.01
pH	6.62	8.98	7.04	6.27	10.17	7.55	6.26	10.77	7.95
Turbidity (NTU)	2.40	52.70	12.27	1.10	47.40	9.32	3.00	86.20	12.16
DO (mg/L)	0.87	12.56	7.80	0.57	17.13	8.29	1.11	18.06	8.07
COD_{Mn} (mg/L)	0.89	3.48	1.48	1.27	2.84	1.87	1.45	3.48	2.21
NH_3-N (mg/L)	0.03	0.39	0.08	0.03	0.30	0.09	0.02	0.73	0.18
NO_3-N (mg/L)	1.08	1.88	1.40	0.57	1.65	1.15	0.08	2.29	1.15
TN (mg/L)	1.28	2.54	1.72	1.12	1.92	1.50	1.42	3.06	1.99
TP (mg/L)	0.01	0.08	0.02	0.01	0.08	0.03	0.01	0.07	0.04
N:P	13:1	254:1	86:1	14:1	192:1	50:1	20:1	306:1	50:1
Velocity (m/s)	0.000	0.081	0.010	0.000	0.086	0.008	0.000	0.027	0.007
Total phytoplankton ($\times 10^5$ cells/L)	8.18	622	85.6	56.3	793	288	30.1	923	284
***Pseudanabaena* sp. ($\times 10^5$ cells/L)**	0.00	242	18.8	0.00	170	47.5	0.00	356	61.8

Table 2. The significance values of the spatial and seasonal differences of the water quality parameters in the three reservoirs.

	Tcells	**Pcells**	**T**	**pH**	**Turbidity**	**DO**	**COD_{Mn}**	**NH_3-N**	**NO_3-N**	**TN**	**TP**
Spatial	0.000	0.000	0.005	0.000	0.015	0.578	0.000	0.000	0.010	0.000	0.000
Seasonal	0.000	0.002	0.000	0.014	0.893	0.000	0.000	0.503	0.001	0.000	0.001

$p < 0.001$.

3.2. Distribution and Dominance of *Pseudanabaena* sp.

Along with the water diversion route, the average depth biomass and frequency of being detected of *Pseudanabaena* sp. increased gradually (Figure 3). At sampling sites #1 and #2, there was no *Pseudanabaena* sp. detected for about four to five months. In regard to the Shiyan Reservoir, *Pseudanabaena* sp. could be detected throughout the entire year. The maximum average depth biomass of *Pseudanabaena* sp. in the cascade reservoirs reached 4.69×10^7 cells/L, which was detected at sampling site #7 in the Shiyan Reservoir during May 2014. The monthly differences of *Pseudanabaena* sp. were also obvious. In the Xili Reservoir, the biomass of *Pseudanabaena* sp. in August 2013 was notably higher than in other months. In the Tiegang Reservoir, the biomass of *Pseudanabaena* sp. remained high during two periods from June 2013 to November 2013, and from February 2014 to April 2014. *Pseudanabaena* sp. occurrences were detected throughout the entire year in the Shiyan Reservoir and were close to or over 1.0×10^7 cells/L. June 2013 and November 2013 were the two months with a relatively higher *Pseudanabaena* sp. biomass. Monthly average biomasses of *Pseudanabaena* sp. of the three reservoirs all exceeded 1.0×10^6 cells/L, which became existing risks for the drinking water safety. *Pseudanabaena* sp. has widely been reported as a MIB producer [14,18,25,31]. The data of the MIB concentrations in the source water of the drinking water plant supplied by the cascade reservoirs were collected and compared with *Pseudanabaena* sp. biomass as shown in Appendix B. The standard limits for the MIB concentration level in drinking water are under 10 ng/L and 9 ng/L in China and the United States [32], respectively. The MIB concentration in the source water was excessive in most conditions. It could be inferred that the proliferation of *Pseudanabaena* sp. was related to the excess of MIB because both had the same varying tendency with each other.

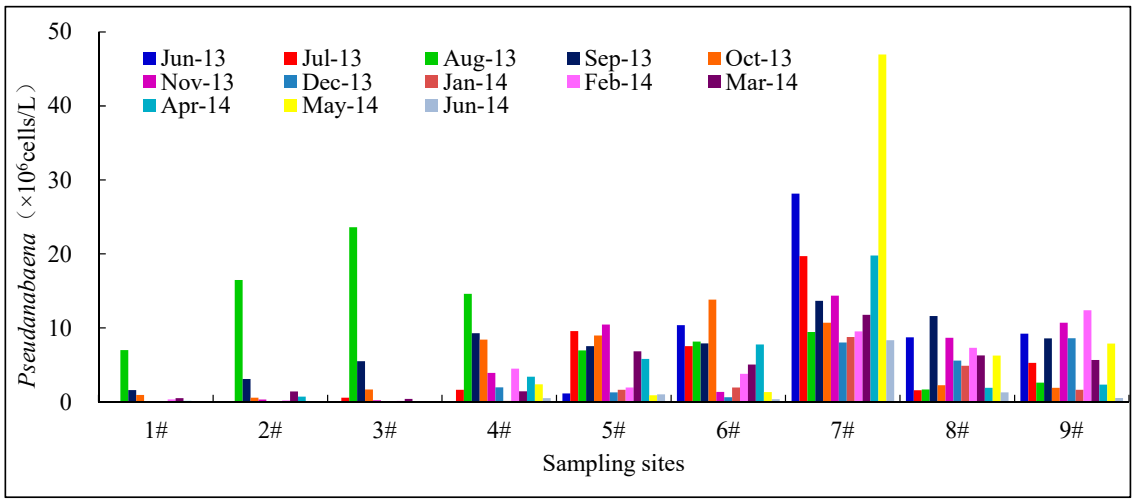

Figure 3. The spatial and temporal distribution of *Pseudanabaena* sp. (depth average).

In this study, the dominance values of *Pseudanabaena* sp. of nine sampling sites were calculated, as shown in Table 3. It is defined that if the value of a species is greater than 0.02, this species is considered dominant throughout the calculating period. Hence, *Pseudanabaena* sp. dominated all of the reservoirs over the whole monitoring period. Moreover, the dominance value was lowest at site #1 and highest at site #7. The *Pseudanabaena* proportion in Figure 4 refers to the ratio of the number of *Pseudanabaena* sp. cells to the total number of phytoplankton cells. In the Xili Reservoir, *Pseudanabaena* sp. was detected in a high proportion from August 2013 to October 2013. The highest *Pseudanabaena* sp. Proportion, which was over 50%, appeared at sampling site #3 in August 2013. The monthly average *Pseudanabaena* sp. proportions of the three sites (#1, #2, and #3) in the Xili Reservoir were 7.57%, 9.15%, and 11.64%, respectively. In the Tiegang Reservoir, although the highest proportion was 40.11% at site #5 in March 2014, which was lower than the Xili Reservoir, the number of months during which the proportion was over 10% increased. Further, there were four months at site #4, five months at site #5, and six months at site #6 with *Pseudanabaena* sp. proportions over 20%. The monthly average *Pseudanabaena* sp. proportions of the three sites in the Tiegang Reservoir (#4, #5, and #6) were 14.91%, 15.69%, and 17.22%, respectively. In the Shiyan Reservoir, the proportion further rose to exceed 30% for most months. There were six months at site #7, four months at site #8, and four months at site #9 during which *Pseudanabaena* sp. proportions exceeded 30%. The highest *Pseudanabaena* sp. proportion reached 53.90% at site #7. The monthly average *Pseudanabaena* sp. proportions of the three sites in the Shiyan Reservoir (#7, #8, and #9) were 26.32%, 22.66%, and 23.53%, respectively. The spatial average *Pseudanabaena* sp. proportion had an obvious seasonal variation, as shown in Appendix C. It dominated mainly in March and August.

Table 3. The dominance value of *Pseudanabaena* sp. in the cascading reservoirs.

Sampling Sites	#1	#2	#3	#4	#5	#6	#7	#8	#9
Dominance value	0.0524	0.0563	0.1075	0.1491	0.1569	0.1722	0.2632	0.2266	0.2353

Recently, *Pseudanabaena* sp. dominance was commonly documented nearly all over the world. For example, several strains of *Pseudanabaena* in two different Southern California reservoirs were isolated in 1998 and were proven to be a common organism in the water from the State Water Project [14]. The tiny filamentous *Pseudanabaena* often appeared in cyanobacterial blooms in brackish and freshwater ecosystems [12]. It also had been dominant in the Hjar reservoir and Znojmo reservoir during the summer season [21,33]. In the Nansi Lake and Dongping Lake of China, *Pseudanabaena* was found to be the dominant species with a maximum proportion of 32.94% [4,19]. The cyanobacterial competition was highly variable, depending on complex biotic and abiotic

conditions [34]. Nevertheless, as previously mentioned, too little attention has been paid to the growth characteristics and influencing factors of *Pseudanabaena* sp.

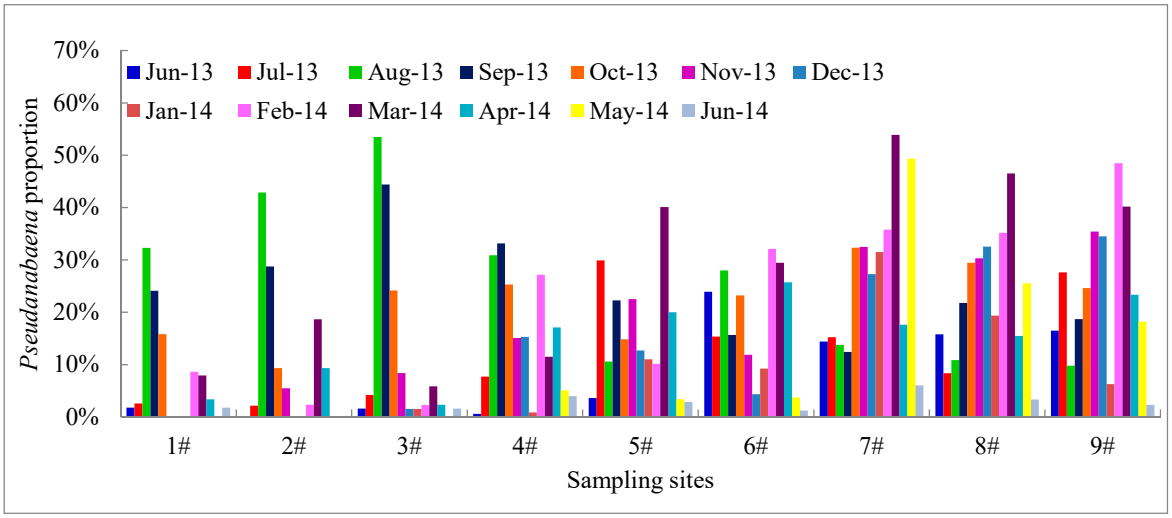

Figure 4. The spatial and temporal distribution of *Pseudanabaena* sp. proportion (depth average).

3.3. The Relationship between Pseudanabaena sp. and Environmental Factors

3.3.1. The Relationship between *Pseudanabaena* sp. and Temperature

Water temperature was acknowledged to be the most important factor contributing to the cyanobacterial abundance. Pearson correlations between *Pseudanabaena* sp. cells/proportion and environment factors were performed, with the results shown in Table 4. *Pseudanabaena* sp. cells were significantly positively correlated with water temperature ($p < 0.01$). The effects of different water temperatures on *Pseudanabaena* sp. growth were further investigated through laboratory experiments. The *Pseudanabaena* sp. growth curve under different water temperatures evaluated by the Chl a concentration is shown in Figure 5. The specific growth rate μ of *Pseudanabaena* sp. on the 16th day was calculated as shown in Table 5. The optimum growth water temperature for *Pseudanabaena* sp. was 25 °C, with the maximum specific growth rate of 0.188 ± 0.001 d^{-1}. It was also found that *Pseudanabaena* sp. has a strong adaptability to temperature. It could grow well with a specific growth rate over 0.13 ± 0.00 d^{-1} under temperatures ranging from 15 °C to 35 °C. However, a temperature within the range from 20 °C to 30 °C was better for the growth of *Pseudanabaena* sp., and this temperature range allowed the specific growth rate to be above 0.16 ± 0.00 d^{-1} on the 16th day. Water temperature was the factor that had the greatest influence on the *Pseudanabaena* sp. distribution. In Nansi Lake of China [19], *Pseudanabaena* is dominant from May to October when the mean water temperature exceeds 20.2 °C, which is consistent with the results of this study. *Microcystis* was limited at temperatures lower than 15 °C and enhanced gradually when the temperature increased up to 25 °C [10,35]. Thus, the preference for the temperature of *Pseudanabaena* sp. was similar to *Microcystis*. In the reservoirs of this study, the monthly mean water temperature of the reservoirs was 25.2 °C (15.3–30.9 °C) for the surface layer, 24.9 °C (15.1 °C–30.7 °C) for the middle layer, and 22.3 °C (14.3 °C–27.7 °C) for the bottom layer, suitable for the *Pseudanabaena* sp. growth. Accordingly, the adaptability of *Pseudanabaena* sp. to a wide range of water temperatures may partly account for its year-round dominance.

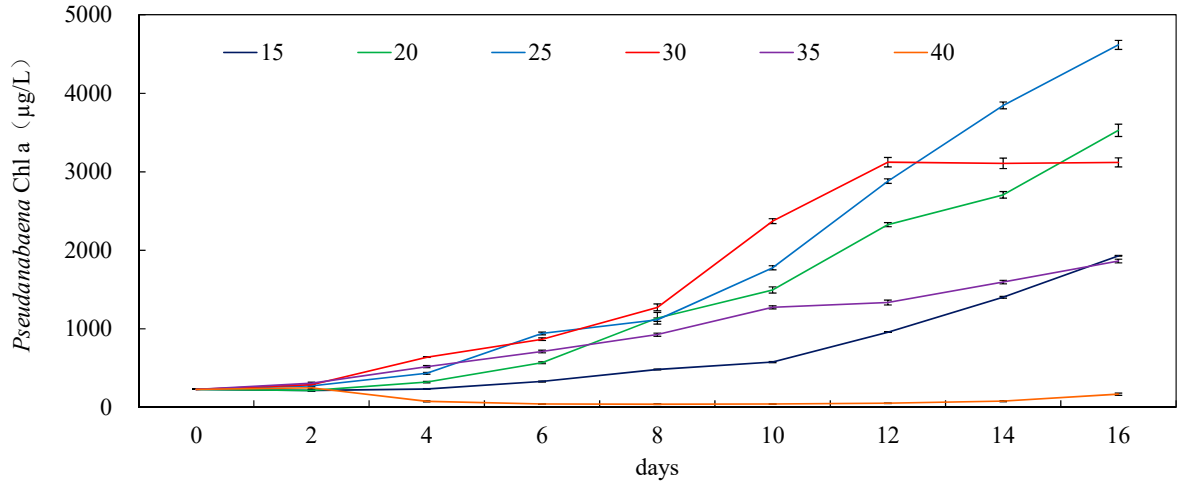

Figure 5. The effects of different temperatures (°C) on *Pseudanabaena* sp. growth.

Table 4. The correlation coefficient analysis between *Pseudanabaena* sp. cells and water quality parameters.

	Secchi Depth	Illumination	T	pH	DO
***Pseudanabaena* (cells/L)**	−0.114	−0.042	0.262 **	0.157 **	0.052
	COD	**NH_3-N**	**NO_3-N**	**TN**	**TP**
***Pseudanabaena* (cells/L)**	0.368 **	0.140 *	−0.187 **	−0.055	0.018

* $p < 0.05$; ** $p < 0.01$.

Table 5. The specific growth rates of *Pseudanabaena* sp. under different environmental conditions.

T (°C)	15	20	25	30	35	40			
μ (d^{-1}) on 16th day	0.13 ± 0.00	0.17 ± 0.00	0.19 ± 0.00	0.16 ± 0.00	0.13 ± 0.00	−0.02 ± 0.01			
Illumination (μmol photons $m^{-2}s^{-1}$)	0.00	9.00	18.00	27.00	36.00	45.00	71.28	142.56	216.00
μ (d^{-1}) on 16th day	−0.13 ± 0.00	0.18 ± 0.00	0.21 ± 0.00	0.23 ± 0.00	0.22 ± 0.00	0.22 ± 0.00	0.22 ± 0.00	0.21 ± 0.00	0.21 ± 0.00
TN (mg/L)	0	2.4	4.8	9.6	19.2	38.4	76.8	153.6	230.4
μ (d^{-1}) on 16th day	−0.11 ± 0.01	−0.01 ± 0.01	0.04 ± 0.01	0.09 ± 0.01	0.17 ± 0.00	0.19 ± 0.00	0.20 ± 0.00	0.21 ± 0.00	0.22 ± 0.00
TP (mg/L)	0	0.04	0.16	0.32	0.64	1.28	5.12	10.24	102.4
μ (d^{-1}) on 16th day	0.06 ± 0.00	0.22 ± 0.00	0.25 ± 0.00	0.27 ± 0.00	0.28 ± 0.00	0.28 ± 0.00	0.24 ± 0.00	0.23 ± 0.00	0.05 ± 0.00
pH without adjustment	3	5	7	8	9	11			
μ (d^{-1}) on 16th day	0.00 ± 0.00	0.21 ± 0.00	0.24 ± 0.00	0.23 ± 0.00	0.24 ± 0.00	0.22 ± 0.00			
pH adjusted to the initial value everyday	3	5	7	8	9	11			
μ (d^{-1}) on 16th day	0.00 ± 0.00	0.00 ± 0.00	0.21 ± 0.00	0.25 ± 0.00	0.25 ± 0.00	0.22 ± 0.00			
Disturbance (r/min)	0	100	200	250	300	400	500		
μ (d^{-1}) on 16th day	0.20 ± 0.00	0.20 ± 0.00	0.21 ± 0.00	0.20 ± 0.00	0.21 ± 0.00	0.21 ± 0.00	0.21 ± 0.00		

One factor was changed while other factors were kept at the following values: temperature was 25 °C, illumination was 71.28 μmol photons $m^{-2}s^{-1}$, the pH was 7, concentrations of TN and TP were 247.00 mg/L and 7.12 mg/L, respectively, no disturbance.

3.3.2. The Relationship between *Pseudanabaena* sp. and Light Intensity

Light intensity is an important factor affecting the growth and photosynthetic activity of *Pseudanabaena* sp. The laboratory experiment results indicated that the increase of light intensity accelerated the growth of *Pseudanabaena* sp. when the illumination was under 27 μmol photons $m^{-2}s^{-1}$, after which a higher light intensity inversely inhibited the growth of *Pseudanabaena* sp. The maximum specific growth rate of *Pseudanabaena* sp. on the 16th day under different light intensities was 0.23 ± 0.00 d^{-1} (Table 5). There is a light saturation point for each cyanobacteria species. When the light intensity reaches the saturation point, the photosynthetic rate of cyanobacteria reaches its maximum and no longer increases with the increase of the light intensity. If the light intensity continues to increase, it may destroy the photosynthetic pigments of cyanobacteria, and the cyanobacteria growth would be inhibited. *Pseudanabaena* sp. is a non-heterocystous cyanobacteria belonging to the order of Oscillatoriales [12]. The Oscillatoriales have commonly been documented to require lower light intensities for growth compared to Microcystis [19,26,36]. In the cascade reservoirs

of this study, the light intensity varied from 0 to 709 µmol photons $m^{-2}s^{-1}$. The average light intensity could reach 142.86 µmol photons $m^{-2}s^{-1}$, more than what *Pseudanabaena* sp. requires. Hence, there was no significant correlation between the *Pseudanabaena* sp. ($p > 0.05$) cells and the illumination in the reservoirs of this study (Table 4).

3.3.3. The Relationship between *Pseudanabaena* sp. and Nutrients

As shown in Table 4, *Pseudanabaena* sp. cells had a positive correlation with ammonium nitrogen ($p < 0.05$) and a significantly negative correlation with nitrate nitrogen ($p < 0.01$). There were no significant correlations between *Pseudanabaena* sp. cells and TN or TP ($p > 0.05$). Growth curves of *Pseudanabaena* sp. were tested under different TN concentrations and TP concentrations in the laboratory. The specific growth rates of *Pseudanabaena* sp. on the 16th day which were calculated by the counts of cells are shown in Table 5. The specific growth rate became distinctly faster following the increase of the initial TN concentration. The maximum specific growth rate reached 0.22 ± 0.00 d^{-1} when the TN concentration was as high as 230.4 mg/L. In this group of experiments, the concentration of TP was 7.12 mg/L in the medium. Conversely, the results of the experiments varying the initial TP concentrations showed that the growth of *Pseudanabaena* sp. was stimulated by increasing the TP concentration when it was under 1.28 mg/L and inhibited when it was over 5.12 mg/L. This reflected the characteristics of the demand for nutrients of *Pseudanabaena* sp. *Pseudanabaena* sp. needs notably more nitrogen than phosphorus according to the report that *Pseudanabaena* sp. was efficient at removing NO_3-N from wastewater that had a high N/P ratio [37]. Moreover, it has been documented that the ammonium uptake rate of several cyanobacteria species was much higher than the nitrate uptake rate [38,39]. The uptake of ammonium may initially be enhanced and the nitrate uptake may be suppressed when N-depleted cyanobacteria are exposed to sudden pulses of inorganic N. The process of uptake and assimilation of NH_4^+ and NO_3^- into intracellular nitrogen pools in macroalgae involves: (1) the uptake across the cell wall, and (2) reduction of NO_3^- to NH_4^+ by nitrate reductase activity [40]. Hence, the utilization of cyanobacteria for nitrogen would give priority to ammonium nitrogen. In the reservoirs of this study, the nitrate nitrogen was the dominant inorganic N source while the concentration of ammonium nitrogen was limited. The nitrate nitrogen and ammonium nitrogen were significantly negatively correlated with each other (-0.153 **, $p < 0.01$). Accordingly, *Pseudanabaena* sp. cells had an opposite correlation with ammonium nitrogen and nitrate nitrogen. The spatial average concentration of TP in the reservoirs ranged from 0.01 to 0.08. According to the results in Table 5, the specific growth rate of *Pseudanabaena* sp. could reach 0.22 ± 0.00 d^{-1} with the TP concentration of 0.04 mg/L. Thus, the phosphorus in the reservoirs was relatively abundant for the growth of *Pseudanabaena* sp. and nitrogen was the main limiting factor.

3.3.4. The Relationship between *Pseudanabaena* sp. and pH and COD

COD was the main factor influencing the growth of *Pseudanabaena limnetica* [24]. Its concentrations in all seasons were strongly correlated with the phytoplankton pigment. The reason for the correlation was inferred to be that the extracellular release of COD from the phytoplankton pigment was an important COD source [41–43]. In the reservoirs of this study, the *Pseudanabaena* sp. cells were significantly positively correlated with COD ($p < 0.01$).

In this study, the monthly average pH value of the three reservoirs increased following the water diversion route and had a significant positive correlation with the *Pseudanabaena* sp. cells ($p < 0.01$). The results of the laboratory experiments showed that the highest specific growth rate on the 16th day of *Pseudanabaena* sp. was in the pH range from 7 to 9 without a pH adjustment (Table 5). In addition, no matter how much the initial pH was, the final pH values were all close to 10 or 11 (Figure 6). In other words, along with the growth of *Pseudanabaena* sp., the cultures became more and more alkaline. In addition, the growth rate could be improved further if the elevated pH was adjusted back to the range from 8 to 9 every day (Table 5). *Pseudanabaena* sp. could not survive under an initial

pH lower than 3 or under a standing pH lower than 5. Thus, the pH partly reflected the growth of *Pseudanabaena* sp. in the reservoirs.

In previous studies, pH was reported to be closely related with the cyanobacteria composition and cyanobacteria biomass [44,45]. It was proven that green algae predominated when the pH was lowered with HCl, and if the pH was raised, the blue-greens would predominate [46]. *Microcystis aeruginosa* has been reported to have the highest growth rate at a pH of 9 [47]. The reason was speculated to involve competition by the cyanobacteria for CO_2. The pH changed the distributions and proportions of inorganic carbon species and thereby affected cyanobacteria growth [45]. Another possibility is that the lower pH simulates cyanophage production and the lysis of the blue-green algae, with a release of nutrients which could be used by the green algae.

Comparing the six experiment groups, growth rates in the pH/TP experiments were obviously higher than other groups. This is because the experiments were conducted in different batches in which the inoculations used were of different growth stages. So, the algae growth rates of different experiments are not comparable, we can only evaluate the impact of a single factor on algae growth.

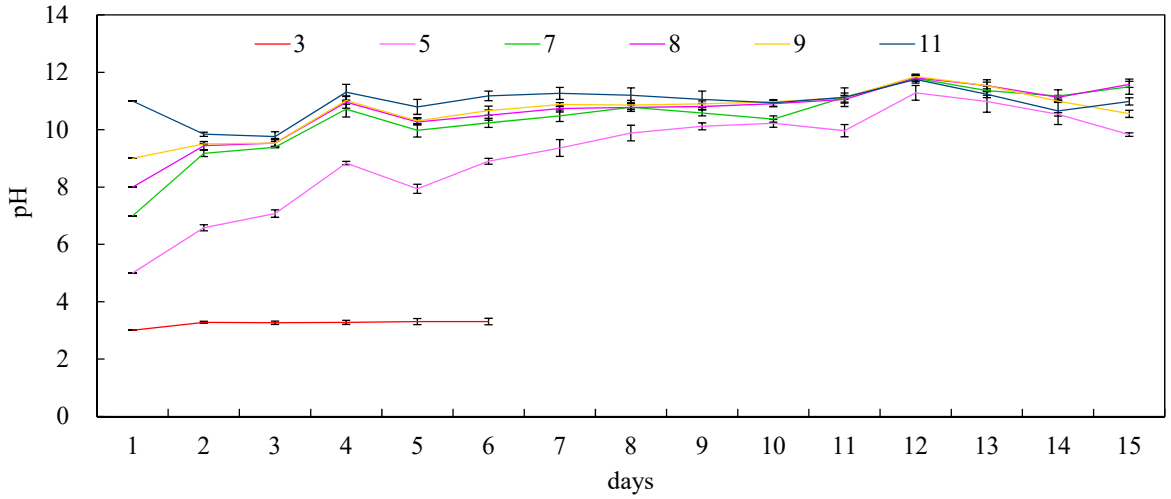

Figure 6. The pH variation following *Pseudanabaena* sp. growth with different initial pH.

3.3.5. The Relationship between *Pseudanabaena* sp. and Disturbance

The effect of disturbance on *Pseudoanabaena* sp. growth was investigated in this study, as shown in Table 5. The specific growth rate on the 16th day under different disturbance intensities showed no obvious differences. An ANOVA was applied to evaluate the growth curves under different disturbance intensities. There were also no significant differences among all of the treatments ($p > 0.05$). Accordingly, disturbance under an intensity of 500 r/min, which simulated a velocity of 0.10 m/s in a lake or reservoir, had no obvious effect on the *Pseudoanabaena* sp. growth. At the same time, mean flow velocities of the reservoirs ranged from 0 to 0.08 m/s. Thus, the mean flow velocities may be another important factor related to the dominance of *Pseudoanabaena* sp. in the cascade reservoirs.

4. Conclusions

Pseudanabaena sp. was found to be dominant throughout the year in the three cascade reservoirs. Evaluating the results of the laboratory experiments and field observations, *Pseudanabaena* sp. had significant positive correlations with water temperature, pH, and COD ($p < 0.01$) and a positive correlation with NH_3-N ($p < 0.05$). The optimum growth temperature range for *Pseudanabaena* sp. was from 20 to 30 °C, hence, it usually outbreaks in May and August. The optimum light intensity and pH for *Pseudanabaena* sp. was 27 μmol photons $m^{-2}s^{-1}$ and from 7 to 9, respectively, and the pH increased with the growth of *Pseudanabaena* sp. The superior tolerance for low light, disturbance, and phosphorus deficiency of *Pseudanabaena* sp. may be the main reason for its dominance in reservoirs.

Nitrogen was the dominant limiting nutrient for *Pseudanabaena* sp. growth. To avoid the risk brought by *Pseudanabaena* sp., nitrogen control was more effective than phosphorus control. This study contributed to the theoretical knowledge for the prediction and control of the growth of *Pseudanabaena* sp.

Author Contributions: Conceptualization, J.G. and J.Z.; Methodology, M.W.; Validation, W.D.; Formal Analysis, J.G.; Investigation, J.G.; Resources, J.G.; Data Curation, J.G.; Writing-Original Draft Preparation, J.G.; Writing-Review & Editing, W.D.; Visualization, J.G.; Supervision, W.D.

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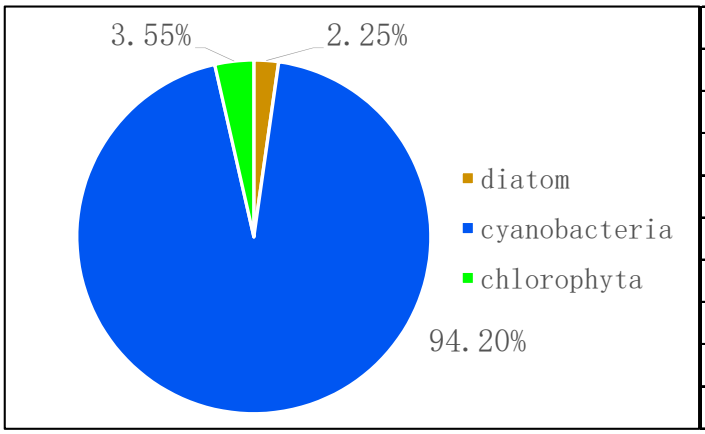
Conflicts of Interest: The authors declare no conflict of interest.

Appendix A



Species	Proportion
Leptolyngbya	63.00%
Raphidiopsis	15.79%
Pseudanabaena	9.05%
Merismopedia	5.07%
Snowella	3.62%
Aphanizomenon	1.81%
Microcystis	0.72%
Chroococcus	0.58%
Anabaena	0.36%

Figure A1. Composition of phytoplankton in the cascading reservoirs and the dominant species.

Appendix B

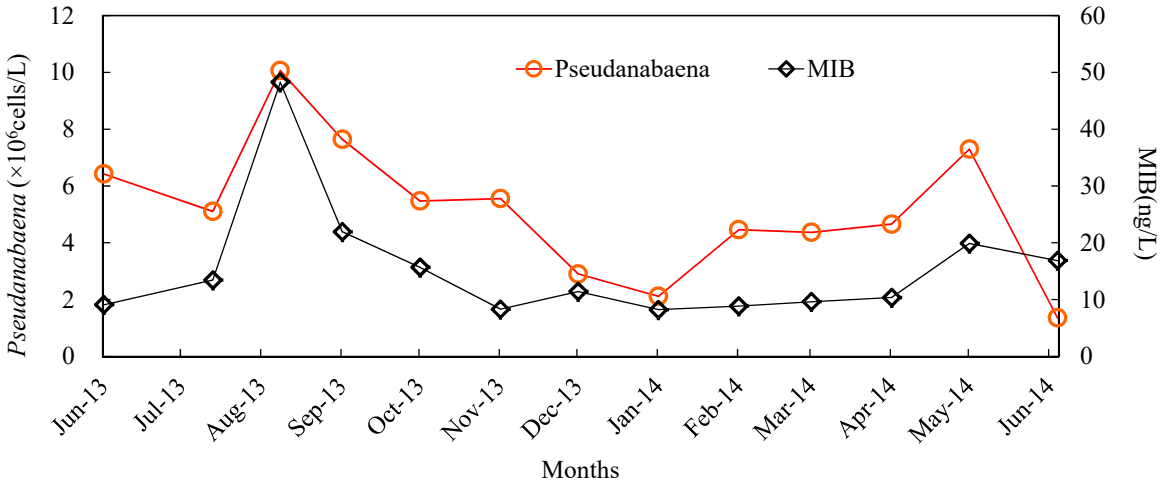


Figure A2. Monthly variations of *Pseudanabaena* sp. and MIB.

Appendix C

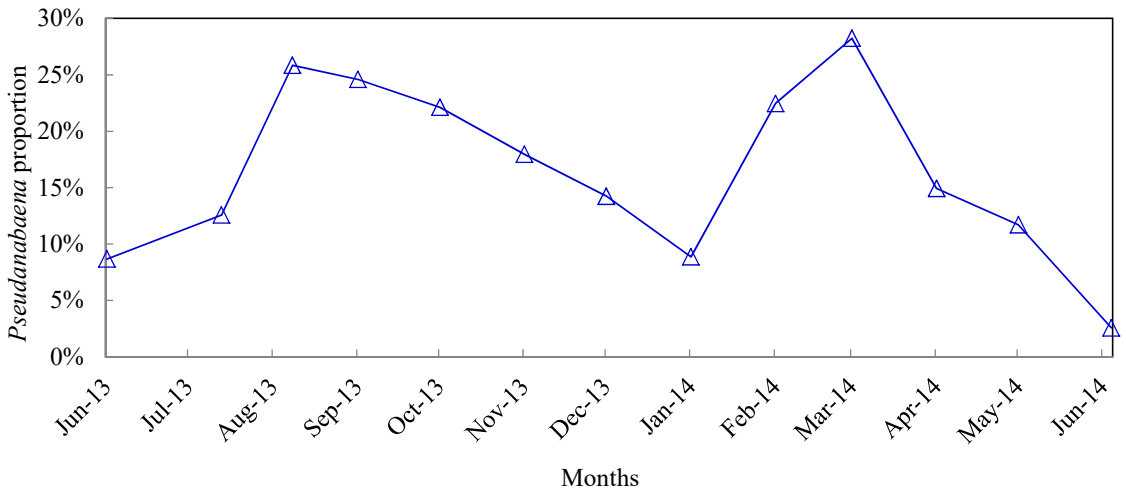


Figure A3. Seasonal variation of *Pseudanabaena* sp. proportion (spatial average).

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